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| <p>(54) Title: IONIC POLYMERS AS ANTI-INFECTIVE AGENTS</p> <p>(57) Abstract</p> <p>A method for treating a microbial infection in a mammal, such as a human, comprising treating the mammal with a therapeutically effective amount of a polymer comprising an amino group or an ammonium group attached to the polymer backbone via an aliphatic spacer group. The polymer can be a homopolymer or a copolymer. In one embodiment, the polymer is a copolymer comprising a monomer having a pendant ammonium group and a hydrophobic monomer.</p> | | |

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IONIC POLYMERS AS ANTI-INFECTIVE AGENTS

BACKGROUND OF THE INVENTION

A number of short (ca. 50 amino acid residues or fewer) linear or cyclic cytotoxic peptides have been isolated recently from a variety of sources. These include mellitin, from bee venom, the magainins, from frog skin, and cecropins, from insects (Maloy, et al., *Biopolymers (Peptide Science)* 37 : 105-122 (1995)). Although of widely varying peptide sequences and structures, these peptides all contain multiple lysine and arginine residues, and, at physiological pH, carry a net positive charge. They also form amphipathic structures wherein one portion of the structure is hydrophilic while the other portion is hydrophobic.

The peptides appear to act solely by direct lysis of the cell membrane (Maloy et al., *supra* (1995)). In the current model, cell lysis is initiated by the electrostatic attraction of the positive charge on the peptide to the negative phosphate head groups at the exterior surface of the membrane phospholipid bilayer. This interaction leads to insertion of the hydrophobic portion of the protein into the membrane, thereby disrupting the membrane structure. The lytic peptides are, in general, more active against prokaryotic cells, such as bacteria and fungi, than eukaryotic cells. This has led to interest in these peptides as potential agents for the treatment of infections in humans (Maloy et al., *supra* (1995); Arrowood et al., *J. Protozool.* 38 : 161S-163S (1991); Haynie et al., *Antimicrob. Agents Chemotherapy* 39 : 301-307 (1995)).

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The natural cytotoxic peptides, however, suffer from several disadvantages with respect to their use as human therapeutic agents. First, it appears that these peptides have evolved to act at high concentration at specific localized sites. Thus, when administered as a drug, the dosage necessary to attain an effective concentration at site of infection can be prohibitively high. A second disadvantage is the difficulty of isolating useful amounts of these peptides from the natural sources, along with the high cost of synthesizing useful amounts of peptides in this size regime. Finally, these compounds, like other peptides, are degraded in the gastrointestinal tract, and, thus, cannot be administered orally.

There is a need for anti-microbial agents which possess the broad activity spectrum of the natural cytotoxic peptides, but are inexpensive to produce, can be administered orally and have lower concentration requirements for therapeutic activity.

20 SUMMARY OF THE INVENTION

One aspect of the present invention is a method for treating a microbial infection in a mammal, comprising administering to the mammal a therapeutically effective amount of a polymer having an amine or ammonium group connected to the polymer backbone by an aliphatic spacer group.

The polymer to be administered can be a homopolymer or a copolymer. In one embodiment, the polymer further includes a monomer comprising a hydrophobic group, such as an aryl group or a normal or branched C₁-C₁₈-alkyl group.

The polymer to be administered can, optionally, further include a monomer comprising a neutral hydrophilic group, such as a hydroxyl group or an amide group.

Another aspect of the invention is a method for treating a microbial infection in a mammal, such as a

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human, comprising administering to the mammal a therapeutically effective amount of a polymer comprising a polymethylene backbone which is interrupted at one or more points by a quaternary ammonium group.

5 The present method has several advantages. For example, the polymers employed are easily prepared using standard techniques of polymer synthesis and inexpensive starting materials. The polymers will not be substantially degraded in the digestive tract and, therefore, can be
10 administered orally. Polymer compositions can also be readily varied, to optimize properties such as solubility or water swellability and antimicrobial activity. Finally, the polymers to be administered include amine or ammonium functional groups attached to the polymer backbone via
15 aliphatic spacer groups. The structural flexibility of such spacer groups minimizes backbone constraints on the interaction of the ammonium groups with anionic targets.

DETAILED DESCRIPTION OF THE INVENTION

20 The present invention relates to a method for preventing or treating a microbial infection in a mammal, such as a human, by administering to the mammal a therapeutically effective amount of a polymer comprising a plurality of amino or ammonium groups which are attached to
25 the polymer backbone via aliphatic spacer groups.

As used herein, a "therapeutically effective amount" is an amount sufficient to inhibit, partially or totally, a microbial infection or to reverse development of a microbial infection or prevent or reduce its further
30 progression. The term "polymer" refers to a macromolecule comprising a plurality of repeat units or monomers. The term includes homopolymers, which are formed from a singly type of monomer, and copolymers, which are formed of two or more different monomers. A "terpolymer" is a copolymer
35 formed from three different monomers. The term polymer, as

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used herein, is intended to exclude proteins, peptides, polypeptides and proteinaceous materials.

As used herein, the term "polymer backbone" or "backbone" refers to that portion of the polymer which is a continuous chain, comprising the bonds which are formed between monomers upon polymerization. The composition of the polymer backbone can be described in terms of the identity of the monomers from which it is formed, without regard to the composition of branches, or side chains, off of the polymer backbone. Thus, a poly(acrylamide) polymer is said to have a poly(acrylamide) backbone, without regard to the substituents on the acrylamide nitrogen atom, which are components of the polymer side chains. A poly(acrylamide-co-styrene) copolymer, for example, is said to have a mixed acrylamide/styrene backbone.

The term "polymer side chain" or "side chain" refers to the portion of a monomer which, following polymerization, forms a branch off of the polymer backbone. In a homopolymer all of the polymer side chains are identical. A copolymer can comprise two or more distinct side chains. When a side chain comprises an ionic unit, for example, the ionic unit depends from, or is a substituent of, the polymer backbone, and is referred to as a "pendant ionic unit". The term "spacer group", as used herein, refers to a polyvalent molecular fragment which is a component of a polymer side chain and connects a pendant moiety to the polymer backbone. The term "aliphatic spacer group" refers to a spacer group which does not include an aromatic unit, such as a phenylene unit.

The term "addition polymer", as used herein, is a polymer formed by the addition of monomers without the consequent release of a small molecule. A common type of addition polymer is formed by polymerizing olefinic monomers, wherein monomers are joined by the formation of a

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carbon-carbon bonds between monomers, without the loss of any atoms which compose the unreacted monomers.

The term "monomer", as used herein, refers to both

- (a) a single molecule comprising one or more polymerizable functional groups prior to or following polymerization, and
5 (b) a repeat unit of a polymer. An unpolymerized monomer capable of addition polymerization, can, for example, comprise an olefinic bond which is lost upon polymerization.

- 10 The quantity of a given polymer to be administered will be determined on an individual basis and will be determined, at least in part, by consideration of the individual's size, the severity of symptoms to be treated and the result sought. The polymer can be administered
15 alone or in a pharmaceutical composition comprising the polymer, an acceptable carrier or diluent and, optionally, one or more additional drugs.

- The polymers can be administered, for example, topically, orally, intranasally, or rectally. The form in
20 which the agent is administered, for example, powder, tablet, capsule, solution, or emulsion, depends in part on the route by which it is administered. The therapeutically effective amount can be administered in a series of doses separated by appropriate time intervals, such as hours.

- 25 Microbial infections which can be treated or prevented by the method of the present invention include bacterial infections, such as infection by Streptococcus, including Streptococcus mutans, Streptococcus salivarius, and Streptococcus sanguis, Salmonella, Campylobacter, including
30 Campylobacter sputum, Actinomyces, including Actinomyces naeslundii and Actinomyces viscosus, Escherichia coli, Clostridium difficile, Staphylococcus, including S. aureus, Shigella, Pseudomonas, including P. aeruginosa, Eikenella corrodens, Actinobacillus actinomycetemcomitans,
35 Bacteroides gingivalis, Capnocytophaga, including

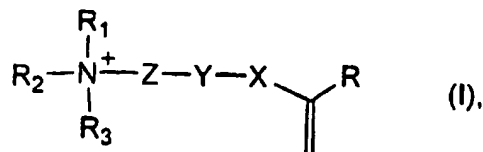
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Capnocytophaga gingivalis, Wolinella recta, Bacteriodes intermedius, Mycoplasma, including Mycoplasma salivarium, Treponema, including Treponema denticola, Peptostreptococcus micros, Bacteriodes forsythus, 5 Fusobacteria, including Fusobacterium nucleatum, Selenomonas sputigena, Bacteriodes fragilis, Enterobacter cloacae and Pneumocystis. Also included are protozoal infections, such as infection by Cryptosporidium parvum and Giardia lamblia; ameobic infections, such as infection by 10 Entamoeba histolytica or Acanthamoeba; fungal infections, such as infections by Candida albicans and Aspergillus fumigatus, and parasitic infections, such as infections by A. castellani and Trichinella spiralis. The method is useful for treating infections of various organs of the 15 body, but is particularly useful for infections of the skin and gastrointestinal tract.

Polymers which are particularly suitable for the present method include polymers which can possess key characteristics of naturally occurring cytotoxic peptides, 20 in particular, the ability to form amphipathic structures. The term "amphipathic", as used herein, describes a three-dimensional structure having discrete hydrophobic and hydrophilic regions. Thus, one portion of the structure interacts favorably with aqueous and other polar media, 25 while another portion of the structure interacts favorably with non-polar media. An amphipathic polymer results from the presence of both hydrophilic and hydrophobic structural elements along the polymer backbone.

In one embodiment, the polymer to be administered 30 polymer comprises a monomer of Formula I,

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wherein X is a covalent bond, a carbonyl group or a CH₂ group, Y is an oxygen atom, an NH group or a CH₂ group, Z is an spacer group, R is a hydrogen atom or a methyl or ethyl group, R₁, R₂ and R₃ are each, independently, a hydrogen atom, a normal or branched, substituted or unsubstituted C₁-C₁₈-alkyl group, an aryl group or an arylalkyl group. Suitable alkyl substituents include halogen atoms, such as fluorine or chlorine atoms.

In the case in which at least one of R₁-R₃ is a hydrogen atom, the monomer can also exist in the free base, or amino form, that is, as the neutral conjugate base of the ammonium cation. The polymer comprising such a monomer can be administered in the protonated, cationic form, such as a salt of a pharmaceutically acceptable acid, or in the free base form. Suitable acids include hydrochloric acid, hydrobromic acid, citric acid, lactic acid, tartaric acid, phosphoric acid, methanesulfonic acid, acetic acid, formic acid, maleic acid, fumaric acid, malic acid, succinic acid, malonic acid, sulfuric acid, L-glutamic acid, L-aspartic acid, pyruvic acid, mucic acid, benzoic acid, glucuronic acid, oxalic acid, ascorbic acid, and acetylglycine. In either case, at physiological pH following administration, a plurality of amino groups will be protonated to become ammonium groups, and the polymer will carry an overall positive charge.

The spacer group is a component of the polymer side chain and connects the amino or ammonium group to the polymer backbone. The amino or ammonium group is, thus, a pendant group. The spacer group can be a normal or

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branched, saturated or unsaturated, substituted or unsubstituted alkylene group, such as a polymethylene group $-(CH_2)_n-$, wherein n is an integer from about 2 to about 15. Suitable examples include the propylene, hexylene and
5 octylene groups. The alkylene group can also, optionally, be interrupted at one or more points by a heteroatom, such as an oxygen, nitrogen (e.g., NH) or sulfur atom. Examples include the oxaalkylene groups $-(CH_2)_2O[(CH_2)_2O]_n(CH_2)_2-$, wherein n is an integer ranging from 0 to about 3.

10 Examples of monomers of Formula I having quaternary ammonium groups include 2-trimethylammonium-ethylmethacrylate, 2-trimethylammoniummethylacrylate, N-(3-trimethylammoniumpropyl)methacrylamide, N-(6-trimethylammoniumhexyl)acrylamide, N-(3-
15 trimethylammoniumpropyl)acrylamide, and N-(4-trimethylammoniumbutyl)allylamine, each of which also includes a counter anion. Examples monomers of Formula I having an amino group include allylamine and N-(3-dimethylaminopropyl)acrylamide.

20 Polymers to be administered which have quaternary ammonium groups or protonated amino groups will further comprise a pharmaceutically acceptable counter anion, such as anions which are conjugate bases of the pharmaceutically acceptable acids discussed above, for example, chloride,
25 bromide, acetate, formate, citrate, ascorbate, sulfate or phosphate. The number of counter anions associated with the polymer prior to administration is the number necessary to balance the electrical charge on the polymer.

The polymer can also be a copolymer further comprising
30 a hydrophobic monomer. The hydrophobic monomer can comprise a side chain bearing a hydrophobic group, such as a straight chain or branched, substituted or unsubstituted C_1 - C_{18} -alkyl group or a substituted or unsubstituted aryl group. Examples of suitable hydrophobic monomers include
35 styrene, N-isopropylacrylamide, N-t-butylacrylamide, N-n-

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butylacrylamide, heptafluorobutylacrylate, N-n-decylallylamine, N-n-decylacrylamide, pentafluorostyrene, n-butylacrylate, t-butylacrylate, n-decylacrylate, N-t-butylmethacrylamide, n-decylmethacrylate, and n-butylmethacrylate.

Examples of copolymers comprising a monomer of Formula I and a hydrophobic monomer include poly(N-(3-dimethylaminopropyl)acrylamide-co-N-(n-butyl)acrylamide) or salts thereof with pharmaceutically acceptable acids.

Other examples of suitable copolymers include poly(2-trimethylammoniummethylemethacrylate-co-styrene) chloride, poly(2-trimethylammoniummethylemethacrylate-co-N-isopropylacrylamide) chloride, poly(2-trimethylammoniummethylemethacrylate-co-heptafluorobutylacryl) chloride, poly(3-trimethylammoniumpropylmethacrylate-co-styrene) chloride, poly(3-trimethylammoniumpropylmethacrylate-co-N-t-butylacrylamide) chloride, poly(3-trimethylammoniumpropylmethacrylate-co-N-n-butylacrylamide) chloride, and poly(N-(3-trimethylammoniumpropyl)allylamine-co-N-n-decylallylamine). Each of these ionic copolymers can also be employed with counter ions other than chloride, for example, a conjugate base of a pharmaceutically acceptable acid.

In a further embodiment, the polymer to be administered comprises a monomer of Formula I, a hydrophobic monomer and a neutral hydrophilic monomer, such as acrylamide, methacrylamide, N-(2-hydroxyethyl)acrylamide or 2-hydroxyethylmethacrylate. Examples of polymers of this type include terpolymers of N-(3-trimethylammoniumpropyl)methacrylamide/N-isopropylacrylamide/2-hydroxyethylmethacrylate, N-(3-trimethylammoniumpropyl)methacrylamide/N-n-decylacrylamide/2-hydroxyethylmethacrylate, N-(3-trimethylammoniumpropyl)methacrylamide/N-t-butylmethacrylamide/methacrylamide, N-(3-trimethylammoniumpropyl)methacrylamide/n-decylacrylate/

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methacrylamide, 2-trimethylammoniummethylemethacrylate/n-butyl-acrylate/acrylamide, 2-trimethylammonium-ethylmethacrylate/t-butylacrylate/acrylamide, 2-trimethylammoniummethylemethacrylate/n-decyl-acrylate/
5 acrylamide, 2-trimethylammonium-ethylmethacrylate/n-decylmethacrylate/methacrylamide, 2-trimethylammonium-ethylmethacrylate/N-t-butyl-methacrylamide/methacrylamide and 2-trimethylammoniummethylemethacrylate/N-n-butyl-methacrylamide/methacrylamide.

10 The polymer to be administered can be an addition polymer having a polymer backbone such as a polyacrylate, polyacrylamide poly(allyl alcohol), poly(vinyl alcohol), poly(vinylamine), poly(allylamine), or polyalkyleneimine backbone. The polymer can have a uniform backbone if it is
15 composed of monomers derived from a common polymerizable unit, such as acrylamide. If the polymer is a copolymer, it can also comprise a mixed backbone, for example, the monomer of Formula I can be an acrylamide derivative, while the hydrophobic monomer can be a styrene derivative. The
20 polymers disclosed herein include examples of both uniform and mixed backbones.

The polymers of use in the present method also include condensation polymers, wherein polymerization of monomers is accompanied by the release of a small molecule, such as
25 a water molecule. Such polymers include, for example, polyesters and polyurethanes.

The polymers of use in the present method are preferably substantially nonbiodegradable and nonabsorbable. That is, the polymers do not substantially break down under
30 physiological conditions into fragments which are absorbable by body tissues. The polymers preferably have a nonhydrolyzable backbone, which is substantially inert under conditions encountered in the target region of the body, such as the gastrointestinal tract.

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The composition of the copolymers to be administered can vary substantially. The copolymer can comprise from about 95 mole percent to about 5 mole percent, preferably from about 20 mole percent to about 80 mole percent, of a monomer of Formula I. The copolymer can also comprise from about 95 mole percent to about 5 mole percent, preferably from about 20 mole percent to about 80 mole percent, of a hydrophobic monomer.

Other examples of polymers which are of use in the present method are disclosed in U.S. Patent Application Serial Nos. 08/482,969, 08/258,477, 08/258,431, 08/469,659 and 08/471,769, the contents of each of which are incorporated herein by reference.

The polymer to be administered will, preferably, be of a molecular weight which is suitable for the intended mode of administration and allows the polymer to reach and remain within the targeted region of the body for a period of time sufficient to interact with the infecting organism. For example, a method for treating an intestinal infection should utilize a polymer of sufficiently high molecular weight to resist absorption, partially or completely, from the gastrointestinal tract into other parts of the body. The polymers can have molecular weights ranging from about 500 Daltons to about 500,000 Daltons, preferably from about 2,000 Daltons to about 150,000 Daltons.

The polymers which are useful in the present method can be prepared by known methods. A first method includes the direct polymerization of a monomer, such as trimethylammoniummethacrylate chloride, or a set of two or more monomers, such as trimethylammoniummethyl-acrylate chloride, N-n-butylacrylamide and acrylamide. This can be accomplished via standard methods of free radical, cationic or anionic polymerization which are well known in the art. Due to reactivity differences between two monomers, the composition of a copolymer produced in this way can differ

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from the composition of the starting mixture. This reactivity difference can also result in a non-random distribution of monomers along the polymer chain.

A second method proceeds via the intermediacy of an activated polymer comprising labile side chains which are readily substituted by a desired side chain. An example of a suitable activated polymer is the succinimide ester of polyacrylic acid, poly(N-acryloyloxysuccinimide) (also referred to hereinafter as "pNAS"), which reacts with nucleophiles such as a primary amine to form a N-substituted polyacrylamide. Another suitable activated polymer is poly(para-nitrophenylacrylate), which react with amine nucleophiles in a similar fashion.

Polymers suitable for use in the present method can also be prepared by addition of a side chain to a preformed polymer. For example, poly(allylamine) can be alkylated at the amino nitrogen by one or more alkylating agents. For example, one fraction of amino groups can be alkylated using a normal or branched C₃-C₁₈-alkyl halide, such as n-decyl bromide, while another fraction can be alkylate by a quaternary ammonium-containing alkyl halide, such as 1-trimethylammonium-4-bromobutane.

A copolymer having a polyacrylamide backbone comprising amide nitrogens bearing two different substituents can be prepared by treating p(NAS) with less than one equivalent (relative to N-acryloyloxysuccinimide monomer) of a first primary amine, producing a poly(N-substituted acrylamide-co-N-acryoyloxysuccinimide) copolymer. Remaining N-acryoyloxysuccinimide monomer can then be reacted with, for example, an excess of a second primary amine to produce a polyacrylamide copolymer having two different N-substituents. A variety of copolymer compositions can, thus, be obtained by treating the activated polymer with different proportions of two or more amines.

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An additional aspect of the present invention is a method for treating a microbial infection in a mammal, such as a human, comprising administering to the mammal a therapeutically effective amount of a polymer having an amino group or an ammonium group within the polymer backbone. The polymer can have, for example, a polymethylene backbone which is interrupted by one or more amino or ammonium groups. An example of a polymer of this type is poly(decamethylenedimethylammonium-co-ethylenedimethylammonium) bromide, which is synthesized via the reaction of N,N,N',N'-tetramethylethylenediamine and 1,10-dibromodecane. The polymer can also be administered in association with anions other than bromide, such as chloride or acetate anions. Other examples include poly(alkyleneimines), for example, poly(ethyleneimine). Such polymers can comprise secondary or tertiary amino groups, salts of such groups with pharmaceutically acceptable acids, and/or quaternary ammonium groups.

As discussed below in Example 35, several polymers described herein were tested for in vitro activity against *Cryptosporidium parvum* infectivity in mammalian cell culture. Of these, poly(TMAEMC-co-styrene), described in Example 7, was most active, exhibiting greater than 90% inhibition of *C. parvum* infectivity relative to the control when applied as a 0.1 mg/mL solution in dimethylsulfoxide. The remaining polymers tested also showed significant anti-*Cryptosporidium* activity.

The invention will now be further and specifically described by the following examples.

EXAMPLES

The following abbreviations are used throughout the examples to denote the following monomers: MAPTAC, N-(3-trimethylammoniumpropyl)methacrylamide chloride; TMAEMC,

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2-trimethylammoniummethacrylate chloride; HEMA, 2-hydroxyethylmethacrylate; TMAEAC, 2-trimethylammonium-ethylacrylate chloride.

The copolymers and terpolymers of the following
5 examples are given nominal compositions which correspond to the molar ratios of starting monomers in the copolymerization mixture.

Example 1 Synthesis of poly(N-acryloyloxysuccinimide)
(pNAS)

10 A solution of N-acryloyloxysuccinimide (25.0 g, 148 mmole) in 100 mL dry DMF was degassed by nitrogen purging and simultaneously heated to 60 °C. To the reaction mixture was added azobisisobutyronitrile (AIBN) (120 mg, 0.005 equivalents with respect to monomer). The reaction
15 was allowed to proceed for 24 hours at 60 °C. The polymer solution was cooled to room temperature and poured into rapidly stirred THF. The resulting white precipitate was filtered, washed with THF and dried in vacuo.

Example 2 Synthesis of poly(N-(3-dimethylamino-
20 propyl)acrylamide-co-N-n-butylacrylamide

To a solution of 3.0 g (17.75 mmole) pNAS in 20 mL dry DMF was added 0.6 g (3.55 mmole) n-butylamine. The resulting solution was stirred at room temperature for 14 hours, and then heated at 60 °C for 4 hours. After the
25 solution was cooled to room temperature, 9.05 g (89 mmole) 3-dimethylaminopropylamine was added, and the resulting solution was stirred at room temperature for 2 hours, then heated to 60 °C for 20 hours. After cooling to room temperature, the solution was diluted with 25 mL water, and
30 dialyzed against water for 24 hours. The solution was then lyophilized to afford poly(N-(3-dimethylaminopropyl-acrylamide)-co-N-n-butylacrylamide) as a tacky white solid.

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Example 3 Synthesis of poly(N-(3-trimethylammoniumpropyl)
acrylamide-co-N-n-butylacrylamide) iodide

To a suspension of poly(3-dimethylaminopropyl-
acrylamide-co-N-n-butylacrylamide in methanol was added 0.5
5 g methyl iodide. The resulting mixture was stirred for 3
hours, and gradually became homogeneous. After stirring
for another 12 hours, the solvent was removed under reduced
pressure and the polymer was washed with dry hexane.

Example 4 Synthesis of poly(N-(2-hydroxyethyl)acrylamide-
10 co-N-(6-trimethylammoniumhexyl)acrylamide)
bromide

To a solution of 2.48 g (15 mmole) pNAS in 5 mL DMF
was added 1.00 g (3 mmole) 1-trimethylammonium-6-hexanamine
bromide. The solution was stirred at room temperature for
15 4 hours and then heated at 60 °C for 20 hours. The
solution was cooled to room temperature, and then 8.95 g
(150 mmole) 2-ethanolamine was added. The resulting
mixture was heated to 80 °C for 20 hours, cooled to room
temperature and diluted with 10 mL water. The solution was
20 dialyzed against water for 24 hours, then lyophilized,
yielding the polymer as a brittle white solid.

Example 5 Synthesis of poly(TMAEAC)

A solution of 48.25 g (0.25 mol) 2-trimethylammonium-
ethylacrylate chloride in 400 mL isopropanol was degassed
25 by nitrogen purging and heated to 35 °C. To this stirred
solution was added a solution of 0.8 g potassium persulfate
in 10 mL distilled water. A slight exotherm was observed.
The solution was stirred at 35 °C for 6 hours, then cooled
to room temperature. The solution was added to hexanes and
30 the resulting precipitate was isolated by filtration.

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Example 6 Synthesis of poly(decamethylenedimethylammonium-co-ethylenedimethylammonium) bromide

N,N,N',N'-tetramethylethylenediamine (10.0 g, Aldrich),
1,10-dibromodecane (25.8 g, Aldrich) and methanol (100 mL)
5 were placed into a three-neck 250 mL round bottom flask.
The mixture was heated with gentle stirring to 65 °C for 6
days, at which point methanol (40 mL) was added, and the
mixture was refluxed for an additional 2 days. The mixture
was then dripped into acetone, forming a solid that was
10 collected by filtration, rinsed with acetone, and dried in
a vacuum oven to yield 30.9 g of product.

Example 7 Synthesis of poly(TMAEMC-co-styrene) 75/25

A 500 mL round bottomed flask was charged with
trimethylammoniummethylemethacrylate chloride (26.0 g of a
15 70wt% aqueous solution, 18.2 g), styrene (6.0 g) and
isopropanol (150 mL). The solution was degassed by the
addition of a rapid stream of nitrogen for 10 minutes,
followed by the addition of AIBN (0.5 g). The solution was
degassed for a further thirty minutes and, while continuing
20 the addition of nitrogen, the solution was heated to 70 °C,
and the temperature maintained for 17 h. The polymer began
to precipitate within 2 h, and by the completion of the
reaction a sticky white precipitate had formed. The
reaction mixture was cooled, the isopropanol was decanted
25 from the polymer, and the polymer was dissolved in
methanol. Dropwise addition of the methanol solution to
ethyl acetate (1200 mL) caused the polymer to precipitate
as a fine white powder which was recovered by filtration.

Example 8 Synthesis of poly(TMAEMC-co-N-isopropyl-
30 acrylamide) (67/33)

A 500 mL round bottomed flask was charged with
trimethylammoniummethylemethacrylate chloride (14.5 g of a
70wt% aqueous solution, 10.0 g), N-isopropylacrylamide

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5.0 g) and isopropanol (150 mL). The solution was degassed by the addition of a rapid stream of nitrogen for 10 minutes, followed by the addition of AIBN (0.5 g). The solution was degassed for a further 60 minutes. The
5 reaction mixture was heated to 70 °C, and the temperature maintained for 16 h. The polymer partially precipitated over the course of the reaction. Upon cooling, the propanol was decanted from the polymer, and the polymer was dissolved in methanol. Precipitation of the methanol
10 solution dropwise into ethyl acetate (1200 mL) caused the polymer to be deposited as white curds which were recovered by filtration, washed with ethyl acetate, and dried in vacuo.

Additional TMAEMC/N-isopropylacrylamide copolymers
15 were prepared by a similar method with the starting monomers in the following ratios:
TMAEMC/N-isopropylacrylamide = 40/60, 25/75 and 15/85.

Example 9 Synthesis of poly(MAPTAC-co-styrene) 75/25

To isopropanol (150 mL) was added a solution of N-(3-
20 trimethylammoniumpropyl)methacrylamide chloride in water (50 wt% solution, 24.0 g, 12.0g of monomer). To this solution was added styrene (6.0 g), followed by the addition of AIBN (0.5 g). The homogeneous solution was degassed by bubbling a stream of nitrogen through it for 30
25 minutes. The solution was heated to 70 °C for 15 h. The polymer partially precipitated as the reaction proceeded. The solution was cooled, the isopropanol was decanted off, the white solid was washed with propanol (50 mL). The propanol was decanted a second time, and the solid was
30 dissolved in methanol (150 mL). The clear solution was added dropwise to ethyl acetate, causing the polymer to be precipitated as a white powder. The polymer was recovered

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by filtration, washed with 50 mL of ethylacetate and air dried.

An additional MAPTAC/styrene copolymer was prepared by a similar method employing a 50/50 mixture of starting
5 monomers.

Example 10 Synthesis of poly(TMAEMC-co-heptafluoro-butylacrylate) 75/25

A 500 mL round bottomed flask was charged with 2-trimethylammoniummethacrylate chloride (26.0 g of a
10 70wt% aqueous solution, 18.2 g), heptafluorobutylacrylate (6.0 g) and isopropanol (150 mL). The solution was degassed by the addition of a rapid stream of nitrogen for 10 minutes, followed by the addition of AIBN (0.5 g). The solution was degassed for a further thirty minutes and,
15 continuing the addition of nitrogen, the solution was heated to 70 °C. The temperature was maintained for 17 h. The polymer began to precipitate within 1 h, and by the completion of the reaction a sticky white precipitate had formed. The reaction mixture was cooled, the propanol was
20 decanted from the polymer, and the polymer was dissolved in methanol (100 mL). Precipitation of the methanol solution dropwise into ethyl acetate (1200 mL) caused the polymer to be deposited as a white solid which was recovered by filtration.

25 Example 11 Synthesis of poly(MAPTAC-co-N-t-butylacrylamide) 75/25

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added
36.4 g of a 50% aqueous solution of N-(3-trimethylammonium-propyl)methacrylamide chloride and 6 g of N-t-butyl-
30 acrylamide followed by 150 mL of isopropanol. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of

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the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The resulting reaction mixture consisted of two phases. The turbid liquid phase was decanted from the bulk of the reaction which was a white sticky solid phase. The liquid was precipitated into 1200 mL of ethyl acetate and filtered by vacuum filtration through a Buchner funnel. The white hygroscopic precipitate was dried in vacuo. The solid phase was dissolved in methanol and precipitated into 1200 mL of ethyl acetate and filtered by vacuum filtration to yield a white powder which was stored under vacuum.

Additional MAPTAC/N-t-butylacrylamide copolymers were prepared by a similar method beginning with the starting monomers in the following ratios: N-(3-trimethylammonium-propyl)methacrylamide/N-t-butyl-acrylamide = 60/40, 50/50, 40/60, and 25/75.

Example 12 Synthesis of poly(N-decylallylamine-co-N-(4-trimethylammoniumbutyl)allylamine)

To a solution of poly(allylamine).HCl (20.15 g of a 50 wt% aqueous solution) was added sodium hydroxide (5.64 g) as a solid. The solution was stirred for 40 minutes, filtered and the filter cake was washed with methanol (15 mL). The solution was further diluted with methanol (25 mL) and to the solution was added 1-bromodecane (7.73 g, 35 mmol) and (1-trimethylamino-4-bromobutane) chloride (9.13 g, 35 mmol). A solution was prepared of sodium hydroxide (2.8 g, 70 mmol) in water (5 mL). This solution was added to the reaction mixture in four portions at thirty minute intervals. The solution was then stirred at room temperature for 24 h, followed by dialysis against deionized water and freeze-dried. A total of 23.2 g of a glassy, hygroscopic solid was recovered.

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Example 13 Synthesis of poly(TMAEMC-co-N-t-butyl-
acrylamide) 57/43

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added
5 18.20 g of a 70% aqueous solution of 2-trimethylammonium-ethylmethacrylic chloride and 9.7 g of N-t-butylacrylamide followed by 150 mL of isopropanol. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of the AIBN
10 dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The resulting reaction mixture consisted of two easily separable phases. The liquid phase was decanted from the bulk of the reaction which was a white solid. The liquid
15 was precipitated into 1200 mL of ethyl acetate and filtered by vacuum filtration through a Buchner funnel. The white precipitate was dried in vacuo and weighed: fraction A, 10.1 g (45.1% yield based on 22.4 g monomers added). The solid phase was dissolved in methanol and precipitated into
20 600 mL of ethyl acetate and filtered by vacuum filtration to yield fraction B, 5.81 g of a white powder (25.9% yield) which was dried under vacuum.

TMAEMC/N-t-Butylacrylamide copolymers were also prepared by a similar method with the starting monomers in
25 the following ratios: TMAEMC/N-t-Butylacrylamide = 63/37, 50/50, 40/60, 25/75, 15/85 and 5/95.

Example 14 Synthesis of poly(MAPTAC-co-N-n-decylacrylamide) 75/25

To a 500 mL round-bottom, three-neck flask fitted with
30 a thermocouple, reflux condenser, and septum was added 36.4 g of a 50% aqueous solution of N-(3-trimethylammonium-propyl)methacrylamide chloride and 6 g of N-n-decylacrylamide followed by 150 mL of isopropanol. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN

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was added. The mixture was purged for ~15 minutes until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The reaction mixture consisted of two easily separable phases. The clear, yellow liquid phase was precipitated into 1200 mL of ethyl acetate. The precipitate was isolated by filtration and dried under vacuum to yield 2.14 g of a yellow powder, fraction A (8.84% yield). Methanol was added to the creamy yellow reaction precipitate and the resulting turbid yellow solution was precipitated into 1200 mL of ethyl acetate. The white precipitate was isolated by filtration and dried under vacuum to yield fraction B, 17.22 g, as a slightly yellow powder (71.2% yield).

Additional MAPTAC/N-n-decylacrylamide copolymers were prepared by a similar method with the starting monomers in the following ratios: MAPTAC/N-n-decylacrylamide = 60/40, 50/50, and 40/60.

Example 15 Synthesis of poly(TMAEMC-co-pentafluorostyrene) 75/25

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 26.0 g of a 70% aqueous solution of 2-trimethylammonium-ethylmethacrylate chloride and 6 g of pentafluorostyrene followed by 150 mL of isopropanol. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The reaction mixture consisted of two phases. The turbid solution was discarded. The bulk of the reaction, consisting of a white solid mass at the bottom of the flask, was dissolved in methanol. The resulting clear solution was precipitated into 1200 mL of ethyl acetate.

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The white precipitate was isolated by vacuum filtration to yield 20.39 g of a fine white powder (84.3% yield).

Additional TMAEMC/pentafluorostyrene copolymers were prepared by a similar method with the starting monomers in the following ratios: TMAEMC/pentafluorostyrene = 60/40 and 50/50.

Example 16 Synthesis of poly(MAPTAC-co-pentafluorostyrene) 75/25

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 36.3 g of a 50% aqueous solution of N-(3-trimethylammonium-propyl)methacrylamide chloride and 6 g of pentafluoro-styrene followed by 150 mL of isopropanol. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The reaction mixture consisted of a turbid solution with a white precipitate. The supernatant was discarded. The white reaction precipitate was dissolved in methanol and the resulting clear solution was precipitated into 1200 mL of ethyl acetate. The white precipitate was isolated by filtration and dried under vacuum to yield 12.81 g of a fine white powder (52.9% yield).

Additional MAPTAC/pentafluorostyrene copolymers were prepared by a similar method with the starting monomers in the following ratios: MAPTAC/pentafluorostyrene = 60/40 and 50/50.

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Example 17 Synthesis of MAPTAC/N-t-Butylacrylamide/
HEMA Terpolymer 33/33/33

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 16.1 g of a 50% aqueous solution of N-(3-trimethylammoniumpropyl) methacrylamide chloride, 8 g of N-t-butylacrylamide, and 8 g of 2-hydroxyethylmethacrylate. The solution was purged with nitrogen for 1 hour and 0.5 g of AIBN was added. The mixture was purged for ~15 min until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The reaction mixture consisted of a turbid solution with a white latex in the bottom of the flask. The solution was precipitated into 1200 mL of ethyl acetate. The white precipitate was isolated by filtration to yield a sticky white powder which was dried under vacuum to yield 10.43 g of a lumpy white solid (fraction A) (43.1% yield). The white reaction precipitate was dissolved in methanol and precipitated into 1200 mL of ethyl acetate. The precipitate was isolated by filtration and dried under vacuum to yield 8.89 g of a fine white powder (fraction B) (36.7% yield).

Additional MAPTAC/N-t-butylacrylamide/HEMA terpolymers were prepared by a similar method beginning with the following ratios of the starting monomers:
MAPTAC/N-t-Butylacrylamide/HEMA = 28/43/28, 23/53/23, and 18/63/18.

Example 18 Synthesis of MAPTAC/N-Isopropylacrylamide/
EMA Terpolymer 18/63/18

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was

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added 150 mL of isopropanol followed by 8.9 g of a 50% aqueous solution of N-(3-trimethylammoniumpropyl) methacrylamide chloride, 15.3 g of N-iso-propylacrylamide, and 4.4 g of 2-hydroxyethylmethacrylate. The solution was
5 purged with nitrogen for 1 hour and 0.5 g of AIBN was added. The mixture was purged for ~15 min until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The clear slightly pink reaction solution was
10 precipitated into 1200 mL of ethyl acetate. The precipitate was isolated by filtration to yield a sticky white solid which was dried under vacuum to yield 14.42 g of a hard clear/white granular solid (59.6% yield).

Example 19 Synthesis of MAPTAC/N-Decylacrylamide/HEMA
15 Terpolymer 33/33/33

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 16.1 g of a 50% aqueous solution of N-(3-trimethylammoniumpropyl)
20 methacrylamide chloride, 8 g of N-decylacrylamide, and 8 g of 2-hydroxyethylmethacrylate. The solution was purged with nitrogen for 1 hour and 0.5 g of AIBN was added. The mixture was purged for ~15 min until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen
25 for 16 hours.

The reaction mixture consisted of two phases. The clear yellow solution was precipitated into 1200 mL of ethyl acetate. The precipitate was isolated by filtration. The sticky yellow precipitate was dried under
30 vacuum and the resulting brittle clear yellow foam was crushed to yield 4.98 g of a fine yellow granular powder (fraction A) (20.6% yield). The white reaction latex was dissolved in methanol and precipitated into 1200 mL of

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ethyl acetate. The precipitate was isolated by filtration and dried under vacuum to yield 10.24 g of a slightly yellow granular solid (fraction B) (42.3% yield).

Additional MAPTAC/N-Decylacrylamide/HEMA terpolymers were prepared by a similar method beginning with the following ratios of starting monomers: MAPTAC/N-Decylacrylamide/HEMA = 28/43/28, 23/53/23, and 18/63/18.

Example 20 Synthesis of TMAEAC/n-Butylacrylate/
crylamide Terpolymer 10/30/60

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 4.84 g of a 50% aqueous solution of 2-trimethylammoniummethacrylate chloride, 7.26 g of n-butylacrylate, and 14.52 g of acrylamide. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The resulting white reaction mixture was filtered by vacuum filtration through a Buchner funnel to yield a white powder. The powder was washed with isopropanol and dried under vacuum to yield 21.57 g of a fine white powder (89.1% yield based on 24.2 g of monomers).

Additional TMAEAC/n-butylacrylate/acrylamide terpolymers were prepared by a similar method beginning with the following ratios of starting monomers: TMAEMC/n-butylacrylate/acrylamide = 20/20/60 and 30/10/60.

Example 21 Synthesis of TMAEAC/t-Butylacrylate/
crylamide Terpolymer 10/30/60

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 4.84 g of a 50% aqueous solution of 2-trimethylammoniummethacrylate

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chloride, 7.26 g of t-butylacrylate, and 14.52 g of acrylamide. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of the AIBN dissolved. The solution
5 was heated to 75 °C under nitrogen for 16 hours.

The resulting white reaction mixture was filtered by vacuum filtration through a Buchner funnel to yield a white powder. The powder was washed with isopropanol and dried under vacuum to yield 21.13 g of a white powder
10 (87.3% yield).

Additional TMAEAC/t-butylacrylate /acrylamide terpolymers were prepared by a similar method beginning with the following ratios of starting monomers: TMAEAC/t-butylacrylate/acrylamide = 20/20/60 and 30/10/60.

15 Example 22 Synthesis of TMAEAC/n-Decylacrylate/
crylamide Terpolymer 10/30/60

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 4.84 g of a 50%
20 aqueous solution of 2-trimethylammoniummethylacrylate chloride, 7.26 g of n-decylacrylate, and 14.52 g of acrylamide. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of the AIBN dissolved. The solution
25 was heated to 75 °C under nitrogen for 16 hours.

The resulting white reaction mixture was filtered by vacuum filtration through a Buchner funnel to yield a white powder. The powder was washed with isopropanol and dried under vacuum to yield 21.52 g of a fine white powder
30 (89% yield).

Additional TMAEAC/n-decylacrylate /acrylamide terpolymers were prepared by a similar method beginning with the following ratios of starting monomers: TMAEAC/n-decylacrylate/acrylamide = 20/20/60, and 30/10/60.

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Example 23 Synthesis of MAPTAC/N-t-Butylmethacryl-
amide/Methacrylamide Terpolymer 10/30/60

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was
5 added 150 mL of isopropanol followed by 4.84 g of a 50% aqueous solution of N-(3-trimethylammoniumpropyl) methacrylamide chloride, 7.26 g of N-t-butylmethacrylamide, and 14.52 g of methacrylamide. The solution was purged with nitrogen for 1 hour and 0.5 g of AIBN was
10 added. The mixture was purged for ~15 min until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The white reaction mixture was too difficult to filter by vacuum filtration so centrifugation techniques
15 were employed instead. The reaction mixture was poured into 50 mL centrifuge tubes and centrifuged. The supernatant was discarded. Isopropanol was added to the polymer and the mixture was stirred and centrifuged. The supernatant was discarded and the white solids were
20 combined and dried under vacuum to yield 14.99 g of a slightly buff powder (61.9% yield).

Additional MAPTAC/N-t-butylmethacrylamide/methacrylamide terpolymers were prepared by a similar method beginning with the following ratios of starting
25 monomers: MAPTAC/N-t-butylmethacrylamide/methacrylamide = 20/20/60, 33/33/33 and 30/10/60.

Example 24 Synthesis of MAPTAC/n-Decylmethacrylate/
Methacrylamide Terpolymer 10/30/60

To a 500 mL round-bottom, three-neck flask fitted
30 with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 4.84 g of a 50% aqueous solution of N-(3-

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trimethylammoniumpropyl)methacrylamide chloride, 7.26 g of n-decylmethacrylate, and 14.52 g of methacrylamide. The solution was purged with nitrogen for 1 hour and 0.5 g of AIBN was added. The mixture was purged for ~15 min until
5 all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The isopropanol was decanted leaving a white chunky powder. Isopropanol was added and the mixture was poured into 50 mL centrifuge tubes and centrifuged. The
10 supernatant was discarded. Isopropanol was added to the polymer and the mixture was stirred and centrifuged. The supernatant was discarded and the white solids were combined and dried under vacuum to yield 18.50 g of a granular white solid (76.4% yield).

15 Additional MAPTAC/N-decylmethacrylamide/methacrylamide terpolymers were prepared by a similar method beginning with the following ratios of starting monomers: MAPTAC/N-decylmethacrylamide/methacrylamide = 20/20/60, 33/33/33 and 30/10/60.

20 Example 25 Synthesis of TMAEMC/n-Decylmethacrylate/
Methacrylamide Terpolymer 10/30/60

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 3.46 g of a 70%
25 aqueous solution of 2-trimethylammoniummethylemethacrylate chloride, 7.26 g of n-decylmethacrylate, and 14.52 g of methacrylamide. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of the AIBN dissolved. The
30 solution was heated to 75 °C under nitrogen for 16 hours.

The white reaction mixture was poured into 50 mL centrifuge tubes and centrifuged. The supernatant was discarded. Isopropanol was added to the polymer and the mixture was stirred and centrifuged. The supernatant was

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discarded and the white solids were combined and dried under vacuum to yield 10.29 g of a hard white solid (42.5% yield).

Additional TMAEMC/N-n-decylmethacrylamide/
5 methacrylamide terpolymers were prepared by a similar method beginning with the following ratios of starting monomers: TMAEMC/N-n-decylmethacrylamide/methacrylamide = 20/20/60, 33/33/33 and 30/10/60.

Example 26 Synthesis of TMAEMC/N-t-Butylmethacryl-
10 amide/Methacrylamide Terpolymer 10/30/60

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 3.46 g of a 70% aqueous solution of 2-trimethylammoniummethacrylate
15 chloride, 7.26 g of N-t-butylmethacrylamide, and 14.52 g of methacrylamide. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16
20 hours.

The white reaction mixture was poured into 50 mL centrifuge tubes and centrifuged. The supernatant was discarded. Isopropanol was added to the polymer and the mixture was stirred and centrifuged. The supernatant was
25 discarded and the white solids were combined and dried under vacuum to yield 18.35 g of a fine white powder (75.8% yield).

Additional TMAEMC/N-t-butylmethacrylamide/
methacrylamide terpolymers were prepared by a similar
30 method beginning with the following ratios of starting monomers: TMAEMC/N-t-butylmethacrylamide/methacrylamide = 20/20/60, 33/33/33 and 30/10/60.

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Example 27 Synthesis of TMAEMC/n-Butylmethacrylate/
Methacrylamide Terpolymer 10/30/60

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 3.46 g of a 70% aqueous solution of 2-trimethylammoniummethacrylate chloride, 7.26 g of n-butylmethacrylate, and 14.52 g of methacrylamide. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The white reaction mixture was poured into 50 mL centrifuge tubes and centrifuged. The supernatant was discarded. Isopropanol was added to the polymer and the mixture was stirred and centrifuged. The supernatant was discarded and the white solids were combined and dried under vacuum to yield 20.99 g of a clumpy white powder (86.7% yield).

Additional TMAEMC/N-n-butylmethacrylamide/methacrylamide terpolymers were prepared by a similar method beginning with the following ratios of starting monomers: TMAEMC/N-n-butylmethacrylamide/methacrylamide = 20/20/60 and 30/10/60.

Example 28 Synthesis of MAPTAC/n-Butylmethacrylate/
Methacrylamide Terpolymer 10/30/60

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 4.84 g of a 50% aqueous solution of N-(3-trimethylammoniumpropyl) methacrylamide chloride, 7.26 g of n-butylmethacrylate, and 14.52 g of methacrylamide. The solution was purged with nitrogen for 1 hour and 0.5 g of AIBN was added. The mixture was purged for ~15 min until all of the AIBN

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dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The white reaction mixture was filtered by vacuum filtration to yield a white powder. The powder was washed
5 with isopropanol and dried under vacuum to yield 22.20 g of a white powder (91.7% yield).

Additional MAPTAC/n-butylmethacrylate/methacrylamide terpolymers were prepared by a similar method beginning with the following ratios of starting monomers: MAPTAC/n-
10 butylmethacrylate/methacrylamide = 20/20/60 and 30/10/60.

Example 29 Synthesis of TMAEAC/n-Decylacrylamide
Acrylamid Terpolymer 33/33/33

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was
15 added 150 mL of isopropanol followed by 16.13 g of a 50% aqueous solution of 2-trimethylammoniummethacrylate chloride, 8.06 g of n-decylacrylamide, and 8.06 g of acrylamide. The solution was purged with nitrogen for 1
20 hour and 0.5 g AIBN was added. The mixture was purged for 15 minutes until all of the AIBN dissolved. The solution was heated to 75°C under nitrogen for 16 hours.

The reaction mixture was precipitated into 1200 mL of ethyl acetate. The fine precipitate was filtered by vacuum filtration to yield a sticky yellow material. The
25 light yellow solid was dissolved in methanol and precipitated into 1200 mL of ethyl acetate. The precipitate was filtered by vacuum filtration to yield 10.85 g of a sticky, slightly yellow powder (44.8% yield).

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Example 30 Synthesis of TMAEAC/N-t-Butylacrylamide/
Acrylamide Terpolymer 33/33/33

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was
5 added 150 mL of isopropanol followed by 16.13 g of a 50% aqueous solution of 2-trimethylammoniummethylacrylate chloride, 8.06 g of N-t-butylacrylamide, and 8.06 g of acrylamide. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for
10 ~15 minutes until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The reaction mixture consisted of a clear colorless solution with a small amount of white sticky solid. The clear solution was precipitated into 1200 mL of ethyl
15 acetate. The white precipitate was filtered, dissolved in water, and lyophilized to yield 6.65 of a white powder (27.5% yield).

Example 31 Synthesis of TMAEAC/Styrene/Acrylamide
Terpolymer 33/33/33

20 To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 16.13 g of a 50% aqueous solution of 2-trimethylammoniummethylacrylate chloride, 8.06 g of styrene, and 8.06 g of acrylamide. The
25 solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The reaction mixture consisted of a clear colorless
30 solution and a white solid. The clear solution was disgarded. The solid was dissolved in methanol, and precipitated into ethyl acetate (1200 mL). A white

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precipitate formed which settled out of the solution as a sticky white solid. The ethyl acetate was decanted and the solid dried by passing nitrogen through the flask. The solid was dissolved in water and lyophilized to yield
5 18.14 g of a fine white powder (75% yield).

Example 32 Synthesis of TMAEAC/n-Butylacrylate/
Acrylamide Terpolymer 33/33/33

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was
10 added 150 mL of isopropanol followed by 16.13 g of a 50% aqueous solution of 2-trimethylammoniummethylacrylate chloride, 8.06 g of n-butylacrylate, and 8.06 g of acrylamide. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for
15 ~15 minutes until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The reaction mixture consisted of a clear colorless solution and a white chunky solid. The solution phase was discarded and the white solid dissolved in water, filtered
20 and lyophilized to yield 12.84 of a fine white powder (53.1% yield).

Example 33 Synthesis of TMAEAC/n-Decylacrylate/
Acrylamide Terpolymer 33/33/33

To a 500 mL round-bottom, three-neck flask fitted
25 with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 16.13 g of a 50% aqueous solution of 2-trimethylammoniummethylacrylate chloride, 8.06 g of n-decylacrylate, and 8.06 g of acrylamide. The solution was purged with nitrogen for 1
30 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

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The white reaction mixture was precipitated into 1200 mL of ethyl acetate. The turbid solution was decanted and the polymer was dried with nitrogen, dissolved in water, and lyophilized to yield 8.79 g of fine white powder (36.3% yield).

Example 34 Synthesis of TMAEAC/t-Butylacrylate/
Acrylamide Terpolymer 33/33/33

To a 500 mL round-bottom, three-neck flask fitted with a thermocouple, reflux condenser, and septum was added 150 mL of isopropanol followed by 16.13 g of a 50% aqueous solution of 2-trimethylammoniummethacrylate chloride, 8.06 g of t-butylacrylate, and 8.06 g of acrylamide. The solution was purged with nitrogen for 1 hour and 0.5 g AIBN was added. The mixture was purged for ~15 minutes until all of the AIBN dissolved. The solution was heated to 75 °C under nitrogen for 16 hours.

The white reaction mixture was precipitated into 1200 mL of ethyl acetate. The turbid solution was decanted and the polymer was dried with nitrogen, dissolved in water, and lyophilized to yield 6.51 g of fine white powder (26.9% yield).

Example 35 In vitro activity of selected polymers
against *C. Parvum* infectivity

Confluent MDBK cell monolayers were grown on 16 well slides, and infected with 5×10^5 of *C. parvum* oocysts per well. Various dilutions of the test reagents in dimethylsulfoxide (DMSO) were added to the monolayers and cultures were incubated at 37 °C (8% CO₂) for 48 hours. The level of *C. parvum* infections was determined and analysed by an indirect immunofluorescence (IF) assay at 48 hours. Anti-*C. parvum* sporozoite rabbit serum (1:1000) was used

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as the primary antibody, and fluorscein-conjugated anti rabbit goat serum (1:100) was used as the secondary antibody. Each dilution was tested in quadruple, and each assay was performed at least two times. The monolayers
5 were methanol fixed and, after IF labelling, the number of parasites observed in 10 high power fields (HPF) per well in each of the four wells per dilution was counted, statistically analysed and compared with infected wells which contained DMSO only. Paromomycin was used as the
10 positive control drug. The results are presented in the following Table.

Table

| Polymer | Concentration (mg/mL) | %Inhibition |
|--|--------------------------|-------------|
| poly(TMAEMC-co-styrene) 25/75, Example 7 | 0.1 | 91.7 |
| | 0.033 | 83.2 |
| | 0.011 | 38.9 |
| | 0.0037 | 3.95 |
| poly(TMAEMC-co-N-t-butylacrylamide), 15/85 Example 13 | 10 | 100 |
| | 1.0 | 100 |
| | 0.1 | 59.1 |
| | 0.01 | 38.0 |
| poly(MAPTAC-co-N-n-decylacrylamide), 40/60 Example 14 | 10 | 100 |
| | 1.0 | 100 |
| | 0.1 | 64.3 |
| | 0.01 | 35.5 |
| poly(MAPTAC-co-N-t-butylacrylamide-co-HEMA) 33/33/33 Example 17 | 10 | 70.2 |
| | 1.0 | 57.4 |
| | 0.1 | 52.1 |
| | 0.01 | 18.4 |
| Poly(TMAEMC-co-heptafluorobutylacrylate 60/40, Example 10 | 0.1 | 91.35 |
| | 0.033 | 53.0 |
| | 0.011 | 23.5 |
| | 0.0037 | 4.2 |
| paromomycin | 2 | 79.4 |

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Equivalents

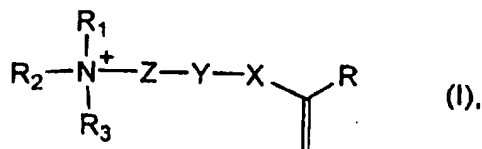
Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific embodiments of the invention
5 described herein. Such equivalents are intended to be encompassed in the scope of the following claims.

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CLAIMS

We claim:

1. A method for treating a microbial infection in a mammal, comprising administering to the mammal a therapeutically effective amount of a polymer having an amino group or an ammonium group connected to the polymer backbone by an aliphatic spacer group.
2. The method of Claim 1 wherein the polymer further comprises a hydrophobic group connected to the polymer backbone.
3. The method of Claim 1 wherein the mammal is a human.
4. The method of Claim 1 wherein the polymer comprises a monomer of Formula I,



15

wherein R is a hydrogen atom or a methyl or ethyl group, X is a covalent bond, a carbonyl group or a CH₂ group, Y is an oxygen atom or an NH or CH₂ group, Z is an aliphatic spacer group, and R₁, R₂ and R₃ are each, independently, a hydrogen atom, a normal or branched, substituted or unsubstituted C₁-C₁₂-alkyl group, aryl or arylalkyl group.

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5. The method of Claim 4 wherein the microbial infection is a bacterial infection, a protozoal infection, an ameobic infection, a fungal infection or a parasitic infection.
- 5 6. The method of Claim 4 wherein Z is a normal or branched C₂-C₁₂-alkylene group or a C₂-C₁₂-alkylene group interrupted at one or more points by a heteroatom.
7. The method of Claim 6 wherein the heteroatom is a
10 nitrogen, oxygen or sulfur atom.
8. The method of Claim 4 wherein at least one of R₁-R₂ is an aryl group, a benzyl group or a normal or branched, substituted or unsubstituted C₁-C₁₈-alkyl group.
- 15 9. The method of Claim 4 wherein the polymer is a homopolymer.
10. The method of Claim 4 wherein the polymer is a copolymer.
11. The method of Claim 10 wherein the copolymer is a
20 terpolymer.
12. The method of Claim 4 wherein the monomer is N-(3-dimethylpropyl)acrylamide, 2-trimethylammoniummethacrylate, 2-trimethylammoniummethacrylate, N-(3-
25 trimethylammoniumpropyl)methacrylamide, N-(6-trimethylammoniumhexyl)acrylamide, N-(3-trimethylammoniumpropyl)acrylamide, or N-(4-trimethylammoniumbutyl)allylamine.

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13. The method of Claim 10 wherein the polymer further comprises a hydrophobic monomer.
14. The method of Claim 13 wherein the hydrophobic monomer has a hydrophobic side chain.
- 5 15. The method of Claim 14 wherein the hydrophobic side chain comprises a straight chain or branched, substituted or unsubstituted C_3 - C_{18} -alkyl group, an aryl group or an aralkyl group.
- 10 16. The method of Claim 15 wherein the hydrophobic monomer is selected from the group consisting of styrene, N-isopropylacrylamide, N-t-butylacrylamide, N-n-butylacrylamide, heptafluorobutylacrylate, N-n-decylallylamine, N-n-decylacrylamide, pentafluorostyrene, n-butylacrylate, t-butylacrylate, 15 n-decylacrylate, N-t-butylmethacrylamide, n-decylmethacrylate, n-butylmethacrylate, n-decylacrylate, and t-butylacrylate.
- 20 17. The method of Claim 15 wherein the polymer comprises at least one monomer selected from the group consisting of N-(3-dimethylpropyl)acrylamide, 2-trimethylammoniummethylemethacrylate, 2-trimethylammoniummethylacrylate, N-(3-trimethylammoniumpropyl)methacrylamide, N-(6-trimethylammoniumhexyl)acrylamide, N-(3-trimethylammoniumpropyl)acrylamide, and 25 N-(4-trimethylammoniumbutyl)allylamine; and at least one monomer selected from the group consisting of styrene, N-isopropylacrylamide, N-t-butylacrylamide, N-n-butylacrylamide, heptafluorobutylacrylate, N-n-decylallylamine, N-n-decylacrylamide, 30

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pentafluorostyrene, n-butylacrylate, t-butylacrylate, n-decylacrylate, N-t-butylmethacrylamide, n-decylmethacrylate, n-butylmethacrylate, n-decylacrylate, and t-butylacrylate.

- 5 18. The method of Claim 13 wherein the polymer further comprises a neutral hydrophilic monomer.
19. The method of Claim 18 wherein the neutral hydrophilic monomer is selected from the group consisting of acrylamide, methacrylamide, N-(2-
10 hydroxyethyl)acrylamide and 2 hydroxyethylmethacrylate.
20. A method for treating a microbial infection in a mammal, comprising administering to the mammal a therapeutically effective amount of a polymer having
15 an amino group or an ammonium group within the polymer backbone.
21. The method of Claim 20 wherein the mammal is a human.
22. The method of Claim 20 wherein the polymer is a poly(alkylene imine).
- 20 23. The method of Claim 20 wherein the polymer is poly(decamethylenedimethylammonium-co-ethylenedimethylammonium) X^- , wherein X^- is an anion, or poly(ethyleneimine).

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24. The method of Claim 20 wherein the microbial infection is an infection by an organism selected from the group consisting of Streptococcus, Salmonella, Campylobacter, Escherichia coli, Clostridium difficile, Staphylococcus, Shigella, Pneumocystis, Cryptosporidium parvum, Giardia lamblia and Entameoba histolytica.
- 5

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/09733

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K31/785

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K C08G

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

8 October 1997

Date of mailing of the international search report

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Name and mailing address of the ISA

European Patent Office, P.B. 5318 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tr. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Gac, G

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 97/09733

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International Application No.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US 97/09733

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

Claims Nos.: 1-3,20,21

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Due to the large number of compounds defined by the claims 1 and 20, the search had to be restricted for economical reasons, and has therefore been performed completely on the compounds as defined by claims 4 and 22 (and dependent claims) and partially (as far as feasible) on the general idea underlying the application (amine or ammonium-containing polymer as therapeutic antimicrobial agent). (PCT Guidelines Chapter III, paragraph 2.3).

Remark : Although claims 1-24 are directed to a method of treatment of the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

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International Application No

PCT/US 97/09733

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